P0414
Paper Poster Session
Diagnosis of emerging viral infections

Evaluation of the modified serotyping-NS1-ELISA to detect dengue NS1 and identify dengue serotypes in patient specimens

Chunya Puttikhunt*[1], Tanapan Prommool[1], Pongpawan Sethanant[2], Narodom Phaenthaisong[1], Nattaya Tangthawornchaikul[1], Panisadee Avirutnan[2], Prida Malasit[2]

[1]*National Science and Technology Development Agency (Nstda), National Center for Genetic Engineering and Biotechnology (Biotec) , Medical Biotechnology Research Unit, Bangkok, Thailand*

[2]*Mahidol University, Faculty of Medicine Siriraj Hospital, Dengue Haemorrhagic Fever Research Unit, Bangkok, Thailand*

Background: Dengue virus (DENV), the cause of the mosquito-borne disease dengue hemorrhagic fever, consists of four serotypes (DENV1, DENV2, DENV3 and DENV4) that co-circulate in endemic areas. Detection of DENV non-structural protein 1 (NS1) in patient blood is one of the most widely used laboratory diagnostics for DENV infection. We had previously established the “Serotyping-NS1-ELISA” to detect DENV NS1 and to identify DENV serotypes simultaneously. However, moderate sensitivity and serotyping errors were found in some specimens especially those from DENV2 infections. This study therefore aimed to modify the new generation of serotyping-NS1-ELISA in order to enhance overall sensitivity and the accuracy of dengue serotyping for all four serotypes.

Material/methods: The serotype-specific biotinylated anti-NS1 MAbs were used to detect NS1 antigens of each serotype which were first captured in microwells with unlabelled, flavivirus cross-reactive MAb 2E11. After enzyme-conjugated streptavidin and substrate addition, the reactivity was measured at 450 nm. Retrospective clinical samples (n=245) from a dengue hospital cohort in Thailand were used to evaluate the assay. Confirmation of dengue infected cases (n=195) and serotype identification were done by RT-PCR. Other febrile illness (OFI), non-dengue cases (n=50) were used as negative controls. The sample dilutions ranged from 1:3 to 1:10.

Results: Five clones of serotype-specific anti-NS1 MAbs, which gave the highest reactivity to NS1 for each serotype and no cross reactivity to others, were selected for this modified assay. Except DENV2-specific clones, either 1B10 or 4B4, the rest of them were the same clones as in the original assay. The detection limit of NS1 for each serotype was lower than 1 ng/ml (compared to 1-15 ng/ml in the original assay). Of 195 dengue infected samples, 156 were positive by either one of five pairs of antibodies and there was no false positive in all non-dengue OFI specimens. The assay specificity was 100% and the overall sensitivity was 80% (compared to 76.5% in the original assay). Sensitivities of the assay for each serotype were 74% for DENV1 (37/50), 82% for DENV2 (41/50), 82.2% for DENV3 (37/45) and 82% for DENV4 (41/50). Importantly, identification of DENV serotypes of those positive samples by our modified assay was 100% accurate for all four serotypes.

Conclusions: We demonstrated that our modified serotyping-NS1-ELISA improved overall sensitivity of NS1 detection as well as its capability to accurately identify all four DENV serotypes. Direct assay of patient plasma in an ELISA format overcomes the complexity of pre-processing specimens prior to serotyping by conventional RT-PCR. This simple serotyping-NS1-ELISA platform is useful for dengue diagnosis and epidemiological studies for which DENV serotype data is necessary.